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Littoral Enclosures for Aquatic Field Testing of Pesticides The Effects of Chlorpyrifos on a Natural System

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16. ABSTRACT

A littoral enclosure protocol designed for aquatic field testing of pesticides is described. The enclosures are replicated, relatively economical, and incorporate all trophic levels, the natural shoreline, and undisturbed bottom sediments. The results of using this protocol to study the environmental chemistry of chlorpyrifos and the effects of chlorpyrifos on the biota in the enclosures are discussed. Chlorpyrifos degradation curves within treatment levels are similar; there was a rapid decline in concentration 1 h after treatment and a slower-than-expected vertical mixing rate occurred. Invertebrate population responses to chlorpyrifos application were generally more severe than would be expected based on laboratory LC_{50} information. Fathead minnow (*Pimephales promelas*) and bluegill (*Lepomis macrochirus*) mortality were similar to predictions based on laboratory data. Significant reductions in growth rates of larval fish appear to be related to dietary changes caused by reductions in chlorpyrifos-sensitive invertebrate forage groups. The results show that this protocol will measure the dissipation, mixing, and persistence of a pesticide, provide information on primary and secondary (ecological) effects on invertebrate and fish populations, and allow a comparison of laboratory toxicity data to actual effects in a natural aquatic system.

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Littoral Enclosures for Aquatic Field Testing of Pesticides: Effects of Chlorpyrifos on a Natural System

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ABSTRACT A littoral enclosure protocol designed for aquatic field testing of pesticides is described. The enclosures are replicated, relatively economical, and incorporate all trophic levels, the natural shoreline, and undisturbed bottom sediments. The results of using this protocol to study the environmental chemistry of chlorpyrifos and the effects of chlorpyrifos on the biota in the enclosures are discussed. Chlorpyrifos degradation curves within treatment levels are similar; there was a rapid decline in concentration 1 h after treatment and a slower-than-expected vertical mixing rate occurred. Invertebrate population responses to chlorpyrifos application were generally more severe than would be expected based on laboratory LC_{50} information. Fathead minnow (*Pimephales promelas*) and bluegill (*Lepomis macrochirus*) mortality were similar to predictions based on laboratory data. Significant reductions in growth rates of larval fish appear to be related to dietary changes caused by reductions in chlorpyrifos-sensitive invertebrate forage groups. The results show that this protocol will measure the dissipation, mixing, and persistence of a pesticide, provide information on primary and secondary (ecological) effects on invertebrate and fish populations, and allow a comparison of laboratory toxicity data to actual effects in a natural aquatic system.

THERE IS A NEED for new and improved procedures to determine primary and secondary (ecological) effects of pesticides on the nontarget biota in natural aquatic systems. This report is a culmination of a 4-yr field validation research effort that began with whole pond studies (Siefert et al. 1984, 1986), evolved to enclosure studies (Siefert et al. 1987), and resulted in the current littoral enclosure design (Brazner et al. 1988).

Pond and enclosure studies have been used to compare results from laboratory tests (Boyle et al. 1985, deNoyelles & Kettle 1985, Larsen et al. 1986), to measure primary and secondary effects of pesticides (Hurlbert et al. 1972, Papst & Boyer 1980, Giddings et al. 1984, Kaushik et al. 1985, Stephenson et al. 1986, Dewey 1986), and to follow the fate and persistence of toxic chemicals (Hurlbert et al. 1970; Stephenson & Kane 1984; Crossland & Wolff 1985; Solomon et al. 1985, 1986).

The above information, experience gained through other aquatic field tests with pesticides at the U.S. Environmental Protection Agency's (EPA) Environmental Research Laboratory–Duluth (ERL-D) and at the University of Wisconsin–Superior, and the field testing requirements of the EPA's Office of Pesticide Programs, pointed to the need to design a new enclosure test system. This system had to (1) be suited for use in ponds or lakes, (2) be relatively simple and economical, (3) be conducive to accurate pesticide environmental chemistry studies, (4) allow for true experimental replication, (5) include the natural undisturbed sediment and shoreline, (6) be representative of the aquatic ecosystem being partitioned, and (7) include habitats affected directly or indirectly by the actual use of a pesticide. Littoral enclosures meet all of these requirements.

Toxicity and environmental chemistry information from field studies is incomplete for most pesticides. Chlorpyrifos (Dursban, Dow Chemical Company, Midland, Mich.) was chosen as a model pesticide for this study because of prior experience in testing chlorpyrifos in the laboratory and in outdoor systems by ERL-D researchers (Holcombe et al. 1982, Jarvinen & Tanner 1982, Jarvinen et al. 1983, Eaton et al. 1984), the availability of numerous laboratory and field studies using chlorpyrifos as a test chemical (Marshall & Roberts 1978), and the high priority status given the pesticide chlorpyrifos by the Office of Pesticide Programs.

The main objectives of the chlorpyrifos chemistry studies were to compare measured pesticide concentrations with the estimated environmental concentration (EEC); determine pesticide dissipation, mixing, and persistence; provide chlorpyrifos concentrations for the biological effects studies. The biological studies were designed to compare laboratory derived toxicity data with changes in field population densities following a pesticide treatment to determine if laboratory toxicity tests predict toxicity accurately in a natural system and to evaluate

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the effectiveness of littoral enclosures and the selected experimental methods for determining direct and indirect (ecological) effects of a pesticide application on aquatic organisms. Although this protocol and the selected methods are not an exhaustive approach to ecosystem analyses, some of the possibilities that could be explored with this system are described.

Materials and Methods

Littoral Enclosures. The littoral enclosures are rectangular (5 × 10 m), bordered with plastic walls on three sides and a 5-m natural shoreline on the fourth side (Fig. 1). The walls are constructed from an 8-mil, woven filament, polyolefin plastic (#888 H UV Clear Scrimweve, Sto-Cote Products, Richmond, Ill.) fastened to untreated wooden snowfence. A complete description and construction guide for littoral enclosures is given by Brazner et al. (1989).

Experimental Design. Twelve enclosures were constructed on the north side of a 2-ha pond located near the Duluth International Airport. The pond has a diverse community of aquatic plants and animals. The littoral areas of the pond are well developed with cattails, pond grasses, and macrophytes growing in unconsolidated, highly organic sediments. Each enclosure included 5 m of shoreline and had an average maximum depth of 1.1 m. Because this kind of enclosure includes natural shoreline, littoral zone, and sediments, all components of the habitat and food supply for endemic pond organisms are available. This design allows replication of a field ecosystem study without seriously compromising the ecological integrity of the results.

Because of the shoreline topography, the enclosures were built in two blocks of six. The two sets of enclosures were separated by approximately 30 m of shoreline. Random assignment of treatment levels to enclosures within each

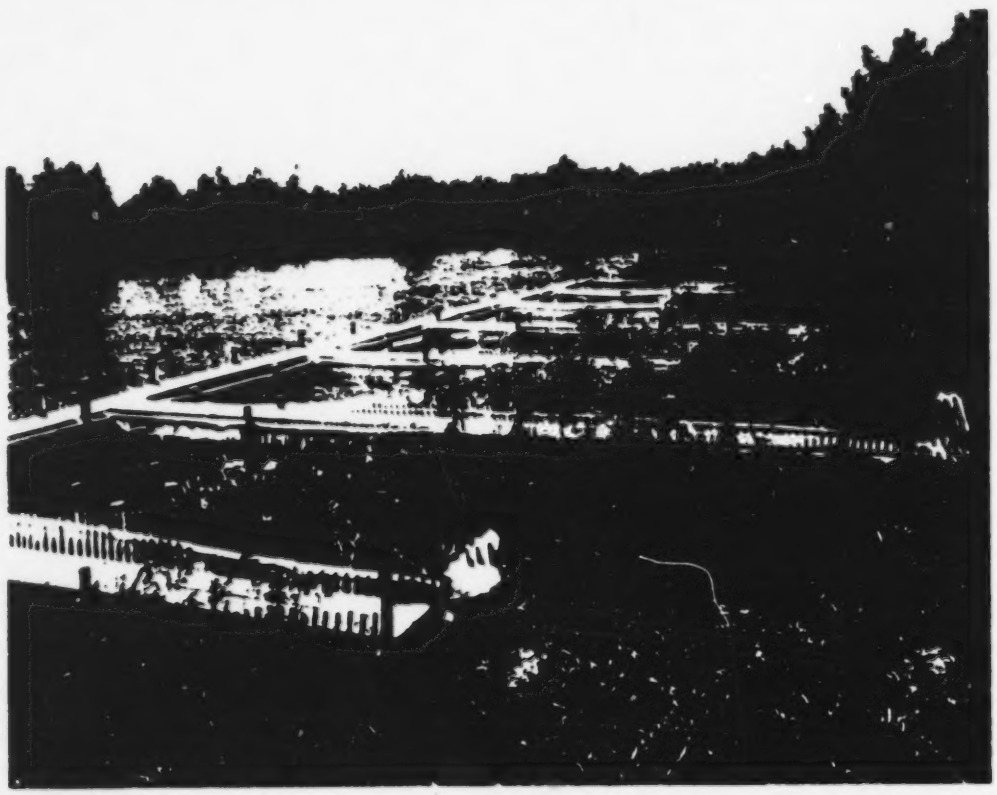

Fig. 1. Littoral enclosures.

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block is described in the enclosure layout below (C, control; L, M, H, low, medium, high treatments):

East Block						West Block					
M*	H*	H	M	L	L*	C	C*	H*	M	M*	H

Fish were introduced to one of the two treatment group enclosures in each enclosure block (denoted by asterisks) before pesticide application. Except for the amount of pesticide applied, the enclosures were treated identically in all other respects. Because there was only one replicate for the control and low treatment groups, these replicates were assigned to the same block. Enclosure dimensions were selected to allow an adequate volume of water and habitat to be enclosed in order to conduct the selected studies and to provide relatively easy replication.

Environmental Chemistry. The water chemistry parameters dissolved oxygen, conductivity, pH, alkalinity, color, turbidity, dissolved organic carbon, dissolved nitrate, and dissolved phosphate were monitored weekly beginning 6 d before pesticide application and through day 64 after treatment in each enclosure. These parameters were used to determine any dose-response relationship between pesticide application and the environmental chemistry of the enclosures. Analyses followed methods of U.S. Environmental Protection Agency (1983a–f, 1984)

Chlorpyrifos Application. Chlorpyrifos [O,O-diethyl O-(3,5,6- trichloro-2-pyridyl) phosphorothioate] in an emulsifiable concentrate Dursban 2E (22.4% AI) was applied on 16 June 1986 at 1000 hours (CDST). The concentrate was mixed thoroughly with 6 liters of distilled water and applied evenly over the enclosure water surface with an 8-liter stainless steel hand sprayer, and a 2.4-m wand. A single application was made to each of the 10 treatment enclosures. Chlorpyrifos concentrations were selected to encompass reported LC_{50} values for a wide variety of pond organisms and the labeled prescribed application rates previously recommended for mosquito control (registration of Dursban 2E for mosquito larvae control was withdrawn November 1985). The application rate to the enclosures was calculated to achieve water concentrations of 20.0, 5.0, and 0.5 μg/liter, respectively. These concentrations required loading rates of 0.123, 0.032, and 0.0029 kg/ha, respectively. The EEC for each treatment concentration was calculated by dividing the pesticide loading (kg) by the weight of water (kg).

Loading from runoff was not used as a criterion to select the concentration ranges for these experiments, which were intended to evaluate a general testing protocol. Runoff estimates are highly variable and depend upon factors such as formulation, rainfall, soil type, extent of buffer zone, and size of watershed. Also, no report of measured chlorpyrifos concentrations resulting from agricultural runoff in natural waters could be found in the literature. However, with the available physical and chemical information on chlorpyrifos combined with recent computer models, it is possible to estimate the loading that could occur from agricultural runoff. Assuming a "typical" situation of average slope, soil type, rainfall, and application rate, and based on the runoff estimates of Wauchope (1978) for the majority of commercial pesticides, a total of 0.5% may be lost to runoff. A typical application rate of 1.12 kg/ha would produce a loading of 0.0056 kg/ha to the aquatic environment. This level is within the selected loading range used in these experiments (0.0029–0.123 kg/ha) and is considered to be reasonable based upon the EPA's extensive experience with pesticide runoff models (Richard Lee, U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C., personal communication).

Water Sampling and Analysis. A composite water sample was collected from each enclosure at predetermined times. Each enclosure was divided by volume into four equal quadrants. A 1-liter sample was taken at approximately mid-depth from each of the four quadrants and combined into 4-liter brown glass solvent bottles for transport and storage. Samples from each treatment enclosure were collected at 1 d before treatment (day −1) and at 1, 2, 4, 8, and 12 h and 1, 2, 4, 8, 16, 32, 64, and 128 d after treatment. Additional samples from the low-treatment enclosure were taken 3 and 5 d after treatment.

To assess the accuracy of the chlorpyrifos analysis procedure, water taken from control enclosures was spiked with chlorpyrifos at the field site. Field and laboratory duplicate samples were analyzed to assess method precision.

The vertical mixing rate of chlorpyrifos after application was investigated in one of the high-treatment enclosures. Water samples were taken starting at a depth of 7.6 cm and at 15-cm depth intervals thereafter to the maximum depth of 100 cm. Samples were taken simultaneously from

each depth at 20-min intervals for 2 h following the pesticide application.

Water samples were extracted in 250-ml volumetric flasks containing 25 ml of isooctane. The sample was shaken and a 25- to 225-ml sample was transferred to the volumetric flask. The flasks were then hand-shaken for 2–3 min and allowed to settle before gas-liquid chromatography analysis. Samples were analyzed for chlorpyrifos on Hewlett-Packard Model 5700 and 5710 gas liquid chromatographs equipped with Ni^{63} electron capture detectors.

Chlorpyrifos Adsorption onto Scrimweve. Laboratory experiments were conducted to evaluate the effect of the plastic barrier material (Scrimweve) on the chlorpyrifos water concentration using experimental methods modified from Sharom & Solomon (1981). An estimate of the chlorpyrifos adsorption to Scrimweve as a function of time and initial chlorpyrifos concentration was obtained.

Plant Studies. Phytoplankton and periphyton biomass and abundance samples were collected with artificial substrates and grab samples to estimate the primary and secondary population responses of plants to pesticide application.

Phytoplankton chlorophyll *a* was used to estimate biomass of algal species. The methods were modified from Wetzel & Likens (1979). A 250-ml polyethylene bottle was lowered to a depth of 30 cm approximately 1 m from the enclosure wall on the deep end and filled with pond water in each enclosure and at open pond sites. The sample was placed on ice and transported to the laboratory.

In the laboratory, the water samples were filtered at 1 atm through a 0.45-μm millipore filter. The filter was placed in an amber jar with 95% acetone and stored overnight. The extract was diluted and measured for fluorescence. Fluorescence was converted to mg/liter chlorophyll *a* from a standard curve. Standards were obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati.

Periphyton biomass was estimated from chlorophyll *a*. An artificial substrate, modified after Stoke's sampler (Stokes 1984) was constructed with Plexiglas, wooden dowels, and Mylar sheets. Three substrates were set 1 m apart in the deeper end of each enclosure and parallel to the outermost walkway. Substrates were collected after 16 d by placing a vial over each submerged dowel. After collection, Mylar sheets were removed from the dowels, put on ice, and sealed with a clean lid. In the laboratory, periphyton was scraped and rinsed from the Mylar sheets. A sample of periphyton was filtered for chlorophyll *a*. Periphyton chlorophyll *a* and enumeration techniques were the same as those for phytoplankton.

Invertebrate Studies. Inverted funnel traps were used to sample zooplankton to determine abundance and composition. This sampler is designed for use in littoral areas (Whiteside 1974) and is set on the substrate where it collects organisms when they move out of the vegetation at night. Samples are relatively detritus free and are easily counted.

Samplers were placed at about sunset in four selected locations in the deeper zones of the enclosures. Filtered pond water (83-μm mesh) was used to fill sample jars. Samplers were collected early the following morning and preserved with 5 ml of 10% formalin. In the laboratory, zooplankton from the three jars were concentrated through an 80-μm screen and identified to genus or species using a compound microscope.

Modified artificial substrate samplers (Hester & Dendy 1962) were used to measure the abundance and composition of macroinvertebrates. This sampler provides clean samples with minimal disturbance to the test system. Samplers were placed in enclosures at a 0.5-m depth contour and allowed to colonize for 16 d. Samplers were collected with an 80-μm net, placed in 0.9-liter (1 qt) freezer containers, and preserved in 10% formalin solution. In the laboratory, samplers were dismantled and each component was washed with tap water through an 80-μm mesh sieve and backwashed into a 125-ml plastic bottle. The 80-μm mesh was used to ensure the collection of chironomid early instars. Samples were represerved in 70% ethanol. Invertebrates were sorted, identified to genus or species, enumerated, and archived in vials containing 70% ethanol. Counts were transformed to log 10 and analyzed by a nested analysis of variance (ANOVA) for differences between populations treated with chlorpyrifos (low, medium, and high) and the control populations.

Primary Effects Fish Studies. In situ bioassays were conducted in 6 of the 12 littoral enclosures (1 control, 1 low, 2 medium, and 2 high treatments) to determine the validity of laboratory fish toxicity data for chlorpyrifos in a controlled field situation. Bluegill sunfish (*Lepomis macrochirus*), a chlorpyrifos-sensitive species with an LC_{50} of about 3.0 μg/liter (Macek et al. 1972,

Johnson & Finley 1980), and fathead minnows (*Pimephales promelas*), a chlorpyrifos-insensitive species with an LC_{50} of 203.0 μg/liter for juveniles (Holcombe et al. 1982), were selected.

Three-month-old bluegills were obtained from Osage Catfisheries in Osage Beach, Mo., and 2- to 3-mo-old fatheads were obtained from the ERL-D Fish Culture Unit. These were transported to the test site in aerated coolers and randomly assigned to test cages after being acclimated to the pond water for 1 h in the coolers.

Stainless steel screen cages (30 cm deep by 60 cm diameter, 0.15-cm square mesh) described by Siefert et al. (1984) were used as test cages. The cages were hung within the six enclosures designed for fish testing so that approximately 3 cm of the cage was above the water line.

Thirty fish of each species were randomly assigned to two cages in each of the fish testing enclosures 3 d before pesticide application. The mean total length of the bluegills was 6.27 cm and their mean wet weight was 4.27 g. The mean total initial length of the fatheads was 4.22 cm and their mean wet weight was 0.6873 g. From the day of assignment until 7 d after treatment (23 June 1986), each cage was supplied with 25–30 g of frozen brine shrimp for food and checked daily for mortality.

Differences in the mean percentage of survival within each treatment group at 4 d after treatment were analyzed by one-way ANOVA on the arcsine-transformed survival percentages (Sokal & Rohlf 1981) followed by Tukey's HSD procedure for comparison of means. The means were based on individual percentage survival values for each cage from all bioassay enclosures of a given treatment type for each species of fish. Field LC_{50} values were determined using the Trimmed Spearman-Karber method (Hamilton et al. 1977). The peak and 24-h weighted average field concentrations were used as the concentrations for the Spearman-Karber calculations. The weighted average was derived from the chlorpyrifos water concentrations measured in the field as described by Siefert et al. (1987).

Secondary Effects Fish Studies. Two studies were performed to determine whether chlorpyrifos concentrations below reported LC_{50} values for a given species of fish could result in changes in the diet and growth rates of these fish due to primary toxicity to more sensitive biota that are nutritionally important forage species. One study used endemic populations of larval fathead minnows that hatched approximately 1 wk before treatment. The other used 30-d-old green sunfish (*Lepomis cyanellus*) that were added to the enclosures from ERL-D Culture Unit stock 3 d before treatment. These two species were selected because their reported LC_{50} values are higher than the chlorpyrifos concentrations expected in the enclosures; both species feed on chlorpyrifos-sensitive invertebrates and both were readily available.

The larval fathead minnows were sampled on days −2, 7, 15, and 31 in each of the 12 enclosures. Sampling dates were scheduled to coincide with zooplankton and macroinvertebrate sampling dates as closely as possible so that digestive tract contents could be compared to available forage. Fish were sampled by pulling a long-handled dip net (25 by 40 cm opening, 500-μm mesh) just under the surface of the water along the enclosure wall. Two 15-m tows were completed in each enclosure on each sample date. Fish were preserved in 10% formalin.

In the laboratory, the number of larval fatheads collected from each enclosure was recorded for each sampling date. Thirty fish were then weighed and measured (standard length) before digestive tract analysis was completed (when total number sampled was < 30, all fish collected were weighed and measured). Species identifications were made when possible and feasible; otherwise, identifications were made to the lowest practical taxonomic group. This was accomplished primarily through the use of keys published by Edmondson (1959), Brooks (1959), Wilson (1959), and Yeatman (1959).

One-way ANOVA was used to test for initial (day −2 samples) and overall density (all samples) differences of larval fatheads among the individual enclosures and among the four treatment groups. Comparisons of mean lengths and weights of larvae from the control and high-treatment enclosures on each of the four sampling dates were made using a nested two-way ANOVA. These analyses were completed using the general linear models procedure (SAS Institute 1982), which included checks for differences associated with block location, treatment group, replicate enclosures within a specific treatment group and block, and any treatment by block interactions.

Analysis of the digestive tract content data was performed using the same form of two-way ANOVA that was used for the within date mean length and weight comparisons followed by Tukey's HSD procedure for comparison of all pairs of treatment means. The means analyzed for the

digestive tract content analysis performed have been based on total counts per fish of each of the major taxa observed in the samples (cladocerans, adult copepods, immature copepods, rotifers, protozoans, chironomids, and immature hydracarina) and for total counts per fish including all the major taxa on a given date.

In the other secondary effects study, 88 30-d-old green sunfish were randomly assigned 3 d before treatment to each of the six enclosures that were used for the fish bioassays. The fish were temporarily held in coolers filled with pond water until assignment was complete and then gradually poured into their assigned enclosures where they could swim unrestricted. During random assignment to the coolers, 30 additional fish were removed for initial length and weight measurements.

Green sunfish were sampled with dip nets or minnow traps, or both, on days −1 and 0 before treatment and days 1, 4, 8, 16, 17, 31, 32, 64, 65, 66, and 67 following treatment. Dip nets were used with equal effort in all enclosures from day 1 through day 8 when the majority of green sunfish were too small to collect with a minnow trap. From day 16 until the end of the study, standard 0.635-cm (0.25-in.) mesh minnow traps were used almost exclusively. Four traps were placed in each enclosure in late afternoon on the day before sampling began and were baited with bread or frozen brine shrimp. Traps were checked by 0900 hours the following morning and sampled fish were put on ice, weighed, and measured at the laboratory and preserved in 10% formalin.

For diet and growth analyses, the samples collected on the dates mentioned above were pooled into three groups: (1) days 16–17, (2) days 31–32, and (3) days 64–67. The length and weight data were analyzed using one-way ANOVA of the means for each treatment group on a given date, followed by Tukey's HSD procedure for comparison of treatment means.

Results and Discussion

Environmental Chemistry. The results of the weekly water chemistry analyses are given in Table 1. In general, most of the changes in water chemistry cannot be attributed to any chlorpyrifos treatment level effect. Conductivity and alkalinity generally increased and turbidity decreased with time during the 134-d study period. The relatively high divergence of some chemical measurements in the east block, high-treatment enclosures, occurred in only one of the enclosures. The variations in this enclosure's measurements are not understood. No significant dissolved oxygen sags occurred during the study in any of the enclosures. Enclosure water temperatures were similar to the pond water temperatures.

Chlorpyrifos Chemistry. The maximum chlorpyrifos concentration in the water was measured 1 h after application in all treated enclosures. Mean concentrations for the low-, medium-, and high-treatment enclosures were 0.51 ± 0.02 (n = 2), 6.29 ± 1.05 (n = 4), and 32.0 ± 5.3 µg/liter (n = 4), respectively. These measured concentrations were higher than the EEC of 0.5, 5.0, and 20.0 µg/liter, respectively. Possible reasons for these differences are the time after treatment and location of initial sampling, the accuracy of the mean depth and surface area measurements used in the calculation of the EEC, the efficiency of the application method, and the pesticide formulation. The 1-h post-treatment values exceeded the EEC by 38, 20, and 2% in the high-, medium-, and low-treatment levels, respectively. By 24 h after treatment, values declined to 69, 73, and 67% of the EEC. This indicates the importance of specifying the initial sample time when comparisons to the EEC are made.

The half-life of chlorpyrifos in the enclosure was inversely proportional to the concentration of the initial treatment. The half-lives for the high, medium, and low treatments were 4–6 h, 8–10 h, and 12–18 h, respectively. The initial rapid decline in chlorpyrifos concentration was followed by a more gradual decline from 12 h to 6 d (Fig. 2).

The persistence of chlorpyrifos was evaluated by the length of time it was detected. The mean chlorpyrifos concentration in the low-treatment enclosures was 0.024 µg/liter at day 8. This represents a 95.5% decrease from the initial peak at 1 h. The concentration was below the quantitation limit (0.01 µg/liter) by day 16. The medium treatment concentration was below the detection limit between 32 and 64 d, and the high concentration became undetectable between 64 and 128 d after treatment.

The chlorpyrifos concentration variability was consistent within treatment levels of this study. The mean coefficients of variation for the high, medium, and low treatments were 21, 16, and 17%, respectively. Chlorpyrifos concentrations at 1 h after treatment ranged from 5.26 to 7.75 µg/liter in the medium and 24.1 to 35.7

Table 1. Averages of weekly water chemistry measurements, 1986 littoral enclosure study

	Enclosures	n	Conductivity, µS/cm	pH	Alkalinity meq/liter (HCO_3)	Color, PCU	Turbidity, NTU	Dissolved organic carbon, mg/liter (C)	Nitrogen, µg/liter (NO_3)	Phosphorus, µg/liter (PO_4)
West Block										
Control	2	24	317.0	8.22	3.46	48.7	2.37	10.6	<1.0	<10.0
Medium	2	24	217.3	8.18	2.48	34.4	1.22	8.8	<1.0	<10.0
High	2	24	223.0	8.14	2.75	39.2	1.30	10.4	<1.0	<10.0
East Block										
Low	2	24	220.0	8.41	2.38	42.9	1.00	11.8	2.0	<10.0
Medium	2	24	296.2	8.14	3.08	55.4	1.07	16.4	57.0	14.2
High	2	24	425.7	8.22	3.27	101.7	1.09	50.4	15,084.0	47.6

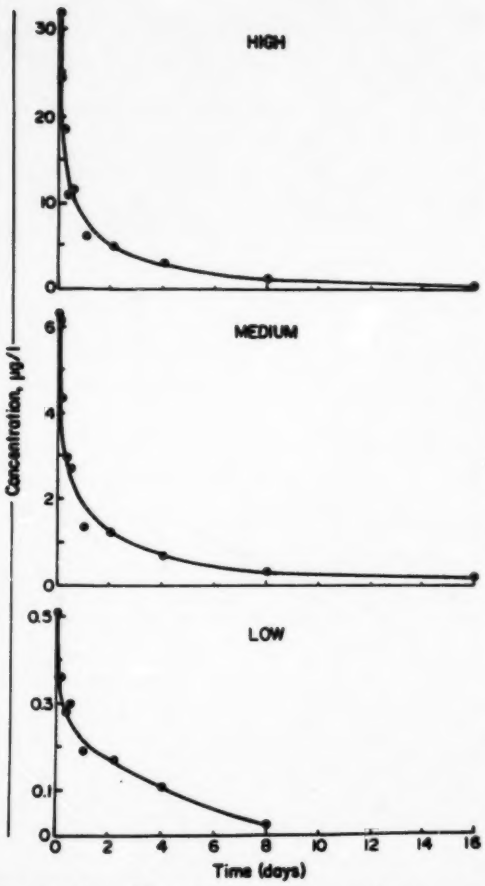

Fig. 2. Mean chlorpyrifos concentrations in low (n = 2), medium (n = 4), and high (n = 4) treatment enclosures, 1986 littoral enclosure study.

µg/liter in the high treatment. The two low-treatment enclosure concentrations were 0.37 and 0.65 µg/liter. The 95% confidence intervals for each treatment did not overlap throughout the study. Chlorpyrifos degradation curves were consistent within and between treatments throughout the 128-d posttreatment period and were comparable to the degradation curve observed in an earlier enclosure study (Siefert et al. 1987). This indicates that pesticide applications to littoral enclosures can be adequately reproduced and that the variability of the pesticide concentration within and between each treatment is consistent.

The vertical mixing rate of chlorpyrifos was investigated in one of the high-treatment enclosures. All of the chlorpyrifos was found in the top 7.6 cm of the water column 20 min after application, and none was detected near the bottom of the water column until 1 h after application. The distribution remained constant for the next hour, and at 2 h 55% of the chlorpyrifos still remained in the top 7.6 cm of water, while 3.6% had reached the bottom.

The mixing rate of chlorpyrifos in the enclosure can have significant implications on the biological and chemical studies. Enclosure water samples taken for pesticide analysis may be biased depending on the sampling method and location and on the distribution pattern of the pesticide. A composite sample of enclosure water for pesticide analysis needs to be integrated over the entire depth to best represent the average water concentration, or sample concentrations should be reported at preselected depth intervals until complete mixing has occurred. Sampling only surface water will yield artificially high concentrations. For valid interpretations, comparisons of field data to

the calculated values of the EEC must specify the sampling technique and time.

The apparent slow mixing rate in the enclosures may also have significant biological implications. Organisms dwelling in the top 10 cm of water may be exposed to higher pesticide concentrations than deeper dwelling organisms. Organisms incapable of migrating may be affected more than those that are more mobile. Fish and invertebrate field bioassays are usually performed in the top 25 cm of water and may result in a higher pesticide exposure than expected.

The accuracy and precision of the chlorpyrifos analysis was monitored throughout the study. The mean recovery of chlorpyrifos from field spikes was 78.3 ± 10.5% (n = 13). The mean relative percentage of difference for laboratory and field duplicate samples was 5.2 ± 5.9 (n = 5) and 12.3 ± 10.0 (n = 10), respectively. The quantitation limit for chlorpyrifos in the water was 0.01 μg/liter.

Adsorption onto the enclosure walls may reduce the pesticide concentration in the water and desorption could prolong the exposure. The enclosure wall material can compete with sediments, plants, and animals for toxicant uptake. In this study, sorption of chlorpyrifos on Scrimweve at an initial concentration of 3.9 μg/liter reached equilibrium in 200 h when 75% of the total chlorpyrifos was adsorbed from the laboratory test system. Assuming equilibrium at 200 h, a second experiment showed that the amount of chlorpyrifos that will adsorb onto Scrimweve is directly proportional to the initial concentration. These data allow the estimate of the maximum adsorption of chlorpyrifos to Scrimweve in the littoral enclosures to be less than 10%. This value represents a maximum or worst case because it assumes a constant chlorpyrifos water concentration for 200 h or more and a clean Scrimweve surface. Neither assumption is valid in the enclosure system because the pesticide concentration was constantly decreasing, and a biological microlayer may inhibit direct adsorption onto the Scrimweve.

Algae. No apparent mortality occurred in the algae community because of chlopyrifos treatment within 4 d after application. The day 4 algae counts were highly variable, with no statistical differences between control and treated enclosures. Approximately 50% of the taxa from the treated enclosures increased as compared with the control populations. There is little freshwater algae acute data, but saltwater algae LC_{50} values for chlorpyrifos range from 138 to 10,000 μg/liter (Maly & Ruber 1983, Borthwick & Walsh 1981). Roberts & Miller (1970) reported reduced growth for unidentified diatoms at a concentration of 40 μg/liter. These values are well above the average initial concentration of 32.1 μg/liter that occurred 1 h after chlorpyrifos application in the high-treatment enclosures. Other field study results indicate that phytoplankton numbers increase after chlorpyrifos application (Butcher et al. 1977, Hughes et al. 1980, Papst & Boyer 1980). This finding is supported by the enclosure study results. By day 8, an algae bloom occurred. This was demonstrated by phytoplankton chlorophyll *a* and periphyton dry weight measurements (Fig. 3). The algal biomass in the treated enclosures was 2–3 times greater than the mean control biomass. By day 16, algal biomass in the treated enclosures was similar to that in the control enclosures. Hurlbert et al. (1972) reported an increase in phytoplankton chlorophyll *a* in chlorpyrifos treated ponds. He suggested that fewer herbivorous rotifers and the elimination of herbivorous crustaceans in treated ponds allowed an increase in growth of algae populations.

Invertebrate Studies. Pretreatment funnel trap sample counts indicated that the microinvertebrate community in early June was dominated by cladocerans, copepods, and rotifers. Cladocerans constituted 44% of the total abundance (112,000/m^2) and copepods 26%. Rotifer populations were also at high densities before treatment and accounted for 22% of the total abundance.

Pretreatment percentage of similarity between control versus low, medium, and high treatment populations was measured by Renkonen's Index (Renkonen 1938) for microinvertebrate funnel trap data. Percentage of similarities for the four treatment groups ranged from 83 to 88%. These values are similar to those observed for microinvertebrates collected from two 50-m enclosures built in a 0.7-ha pond in central Minnesota (Siefert et al. 1987). Brock (1977) assumed for pollution studies that a percentage of similarity of 85% or higher would be expected for two stream communities that are structurally similar. In the littoral enclosures, the percentage of similarity declined to 74–78% 4 d after pesticide application, indicating that the species composition had changed.

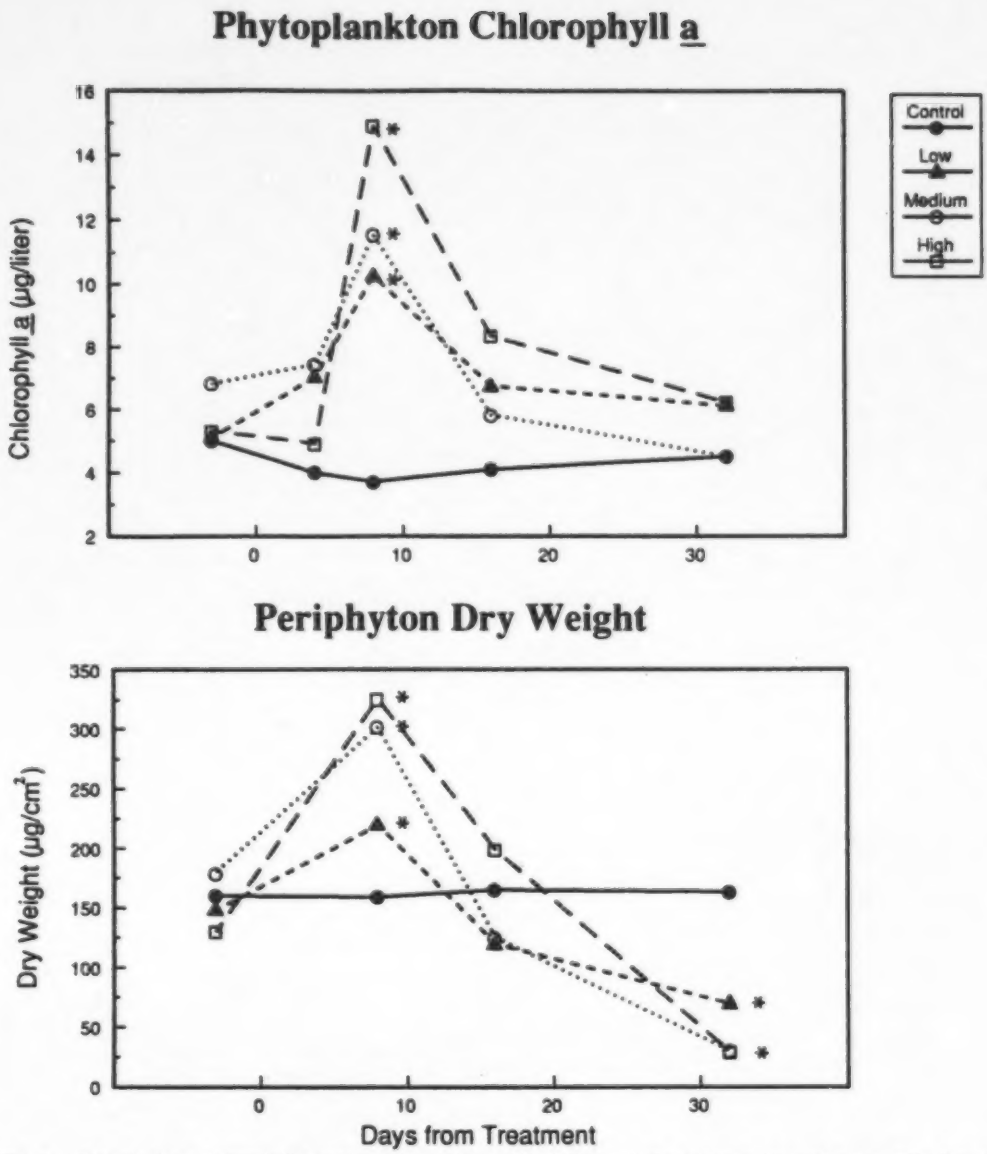

Fig. 3. Phytoplankton chlorophyll *a* and periphyton dry weight before and after chlorpyrifos application, 1986 littoral enclosure study. Asterisks indicate significant difference ($P = 0.05$) from controls.

Invertebrate abundance within the treated enclosures declined compared with that of the control populations. The microinvertebrate community on day 4 markedly differed from the control populations at all three treatment levels, showing a decline of 30 of 35 taxa as compared with the control populations (Table 2). Total abundance of all treated populations was reduced as compared with the control populations. Cladocerans and ostracod populations declined markedly in the treatment enclosures, with a complete loss of *Pleuroxus* in the high-treatment enclosures. Except for *Acanthocyclops* and *Monostyla*, the treated co-

Table 2. Estimated microinvertebrate abundance given as geometric means of organism numbers per m², treatment and control enclosures, day 4, 1986 littoral enclosure study

Taxa	Control $n = 2$	Low $n = 2$	Medium $n = 4$	High $n = 4$	Standard deviation[c]
Cladocerans					
Chydorus sphericus	18,305	123*	36*	23*	0.279
Pleuroxus spp.	1,160	12*	4*	0*	0.178
Simocephalus spp.	1,475	41*	4*	1*	0.254
Alona spp.	749	181*	4*	1*	0.142
Ceriodaphnia	1,323	4*	0*	1*	0.199
Total cladocerans	23,012	361	48	26	—
Copepoda					
Acanthocyclops vernalis	2,689	414	111*	48*	0.363
Macrocyclops	1,843	200	768	414	0.344
Eucyclops spp.	1,349	374	420	261	0.492
Nauplii	10,749	2,269	4,395	2,728	0.255
Copepodites	9,117	1,422	2,169	1,202	0.431
Harpacticoid[a]	2,616	288*	2,197	1,973	0.181
Unknown Cyclopoid sp.[a]	2,785	345	1,041	157*	0.342
Total copepods	31,148	5,312	11,101	6,783	—
Ostracoda					
Cyclocypris	2,649	568*	355*	113*	0.142
Rotifera					
Brachionus	1,172	371	917	708	0.320
Platyias spp.[a]	1,589	51*	310	350	0.284
Monostyla	1,542	117*	394	224*	0.258
Polyarthra	157	66	722	369	0.329
Trichocerca spp.	1,941	634	1,571	687	0.277
Keratella spp.	2,945	529	1,602	1,224	0.392
Testudinella[a]	667	127*	594	517	0.170
Notholca	2,896	1,098	994	1,290	0.386
Lecane[a]	123	11*	26*	45	0.176
Lepadella	5	32	69	30	0.287
Asplanchna[a]	105	0*	45	71	0.239
Euchlanis	2,424	183	774	445	0.418
Trichotria	322	235	151	225	0.243
Ploesoma[a]	101	143	85	51	0.482
Mytillina	408	173	181	101	0.386
Unknown sp.	994	1,011	2,673	1,126	0.320
Total rotifers	17,391	4,781	11,108	7,463	—
Other Invertebrates					
Amoeba[a]	536	1,863*	1,029	1,444*	0.146
Planaria	3,729	2,343	6,417	2,882	0.422
Total organisms	78,465	15,228	30,058	18,711	—

*, $P \leq 0.05$, Tukey's Studentized Range Test.
[c]The SD between means was derived from the ANOVA pooled mean squared error.

pepod and rotifer populations were not statistically different from the control populations.

During the study period, 91 taxa of macroinvertebrates were found in the artificial substrates. The sample sizes of 18 taxa from these substrates were adequate to allow analysis of variance to compare the control macroinvertebrate populations with the populations in the treated enclosures. The macroinvertebrate community in the control enclosure was dominated by chironomids (83% of total abundance) on day 4, the first day of artificial substrate sampling (Table 3). Total abundance of the treated populations was reduced by at least 65% compared with the control populations. Except for snails and planarians, all treated populations of macroinvertebrates were lower than populations in the control enclosures. The most sensitive organisms to chlorpyrifos were the chironomids and amphipods. Chironomids declined to 10% of the control abundance in the high-treatment enclosures and the amphipods declined to 20% of the control abundance.

The funnel traps and artificial substrate samplers worked well for monitoring the population responses of invertebrates to chlorpyrifos. Fun-

Table 3. Estimated macroinvertebrate abundance given as geometric means of organism numbers per artificial substrate, treatment and control enclosures, day 4, 1986 littoral enclosure study

Taxa	Control n = 2	Low n = 2	Medium n = 4	High n = 4	Standard deviation[a]
Chironomidae					
Ablabesmyia	12.2	4.2*	0.4*	0.9*	0.119
Psectrocladius	15.2	0.3*	0.3*	0.3*	0.009
Brillia	7.8	0.1*	0.0*	0.2*	0.105
Corynoneura	14.1	1.6*	0.3*	0.8*	0.176
Polypedilum	3.0	0.4*	0.2*	0.1*	0.088
Glyptotendipes	29.0	10.3	1.4*	1.5*	0.186
Endochironomus	48.2	0.6*	1.0*	0.3*	0.155
Paratanytarsus	53.4	8.4*	1.2*	0.4*	0.221
Tanytarsus	5.8	0.5*	0.0*	0.1*	0.036
Pupae	6.6	1.6*	0.7*	0.3*	0.167
Total	209.5	32.5	5 6	4.9	0.059
Other insects					
Palpomyia	8.3	1.3*	3.1*	1.0*	0.086
Caenis	31.6	7.6*	12.1*	4.9*	0.165
Amphipoda					
Hyalella azteca	14.3	2.5	0.3*	0.0*	0.316
Arachnida					
Hydracarina	55.5	22.1	53.3	39.4	0.100
Other Invertebrates					
Nais	28.2	17.7	26.9	23.4	—
Hydra	78.4	55.6	39.1	41.9	0.129
Physella	5.3	4.0	8.5	4.7	0.203
Planaria	48.8	33.7	59.1*	55.4*	0.163
Total number of organisms	431.1	143.3	148.9	120.2	—

*, $P \leq 0.05$, Tukey's Studentized Range Test, $P < 0.05$.
[a]The SD between means was derived from the ANOVA pooled mean squared error.

nel traps were efficient in collecting littoral area species of cladocerans, copepods, ostracods, and rotifers (microinvertebrates). Whiteside & Williams (1975) reported sampling efficiencies of 90 to 94% for total chydorid abundance using overnight funnel traps within littoral areas in Lake Itasca, Minn. Brakke (1976) found no significant differences in composition of microcrustaceans collected in either funnel traps or large volume net samples. The day 4 estimates of 20,214 chydorids/m^2 (*Chydorus*, *Pleuroxus*, and *Alona*) (Table 2) are similar in magnitude to the estimates of Whiteside & Williams (1975) and Brakke (1976) of 20,000 to 50,000 chydorids/m^2. For artificial substrates it is more difficult to make comparisons with other studies because exposure time and sampler construction vary between studies. However, the artificial substrates were successful in demonstrating treatment differences (Table 3).

Laboratory to field comparisons were made by plotting 24- to 96-h LC_{50} laboratory tests from the literature with day 4 enclosure invertebrate population data (Fig. 4). The end point for the enclosure data was the lowest observed effect concentration (LOEC). The LOEC is reached when the treated populations are statistically different (reduced in numbers) from the control populations.

Each of the bracketed areas for the high, medium, and low chlorpyrifos treatments in Fig. 4 is bordered by the initial 1-h peak measurement and the lower 24-h weighted average concentration. Because chlorpyrifos rapidly dissipates from the water column (Fig. 2), the lower 24-h weighted average concentration may be the most accurate concentration to compare with the laboratory water concentrations. At the low enclosure treatment concentration, 19 of the 48 taxa of invertebrates were significantly reduced in numbers at day 4. Assuming that an LC_{50} represents an ecologically significant response, this population reduction is more severe than would be predicted by acute LC_{50} data. Based on the total reported LC_{50} values, it would be anticipated that the low-enclosure treatment would have nine taxa populations that would be significantly lower than control populations. For direct comparisons, there are only a few enclosure-tested taxa that have laboratory acute val-

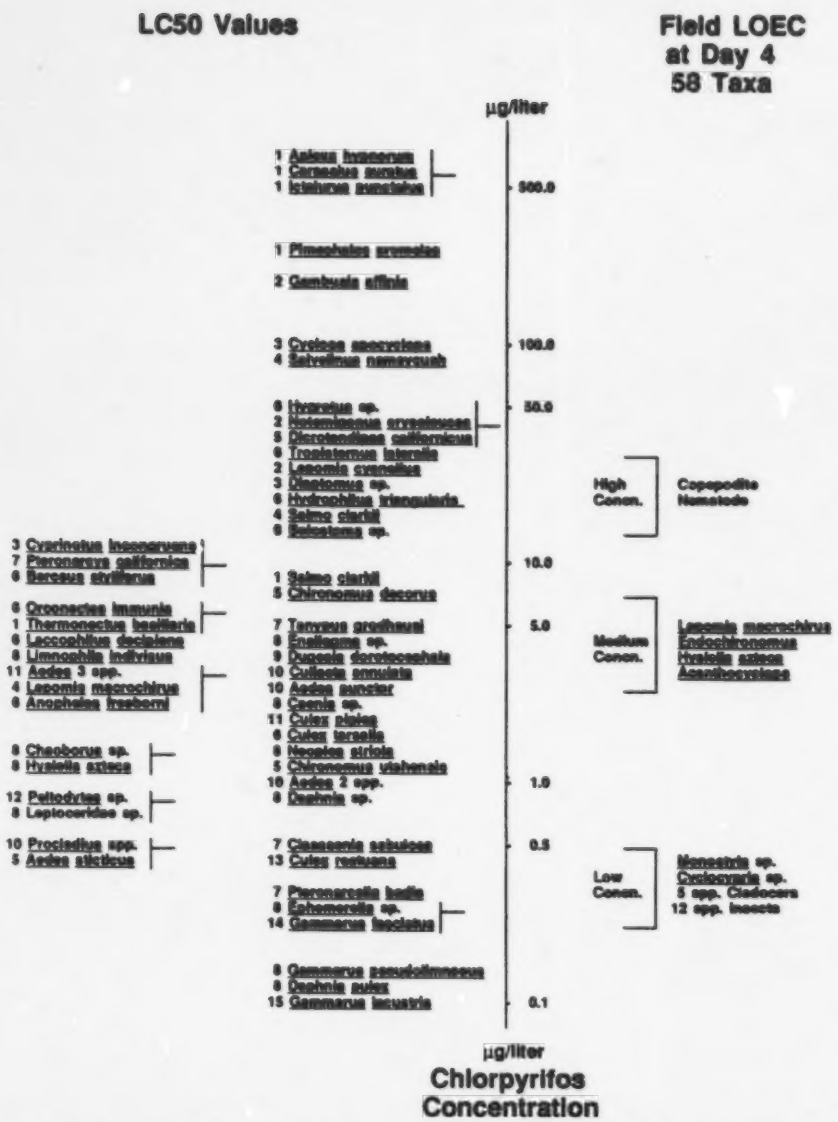

Fig. 4. Comparison of chlorpyrifos LC_{50} values and field (enclosures) lowest observed effect concentration (LOEC), 1986 littoral enclosure study. The high bracket arrow for each treatment denotes the maximum measured chlorpyrifos concentration; the low bracket arrow denotes the 24-h weighted average concentration. Only taxa with populations significantly reduced from control populations have LOECs included. Numbers before names of taxa denote sources for LC_{50} values: 1, Phipps & Holcombe 1985; 2, Ferguson et al. 1966; 3, Ruber & Baskar 1969; 4, Johnson & Finley 1980; 5, Ali & Mulla 1978; 6, Ahmed 1976; 7, Sanders & Cope 1968; 8, Siefert et al. 1987; 9, Levy & Miller 1978; 10, Rettich 1977; 11, Saleh et al. 1981; 12, Federle & Collins 1976; 13, Helson et al. 1979; 14, Sanders 1972; 15, Sanders 1969.

ues. The mayfly *Caenis* and the bluegill *Lepomis macrochirus* had LOECs at or below the laboratory LC_{50} values. A midge, *Dicrotendipes* sp., had a markedly lower LOEC (0.5 μg/liter) than the LC_{50} for *D. californicus* (35 μg/liter). *Hyalella azteca* was the only invertebrate with a higher LOEC than LC_{50} value (1.3 μg/liter). However, at the low field concentration (0.5 μg/liter), the population of *H. azteca* was reduced to 19% of the control population. Although not statistically significant ($P \leq 0.09$), this response could be ecologically significant because this organism is an important food item for fish (Cooper 1965, Strong 1973).

Fish Studies. The results of the caged fish bioassays were similar to predictions based on the 96-h laboratory LC_{50} values reported for these species and the expected littoral enclosure concentrations. The differences in mean percentage of survival observed 4 d after application were not statistically significant for fathead minnows in any of the four treatment groups (Table 4). However, mean percentage of bluegill survival in the control (100%) and low (98.3%) treatment enclosures 4 d after treatment was significantly higher than in the medium (62.2%) and high-treatment (0.9%) enclosures.

The 96-h littoral enclosure field LC_{50} calculated for bluegills was 7.24 μg/liter (95% CL, 5.94–8.83 μg/liter) using the peak chlorpyrifos concentrations and 2.67 μg/liter (95% CL, 2.21–3.22 μg/liter) using the 24-h weighted average chlorpyrifos concentrations. Because chlorpyrifos degrades rapidly in a natural aquatic system, an LC_{50} value based on the peak concentrations might be misleading. Based on laboratory pulse–dose exposure studies (Jarvinen et al. 1988), the effects (EC_{50}) resulting from the first 24 h of exposure to chlorpyrifos are about the same as those observed from a 96-h exposure at the same concentration. Therefore, it seems more reasonable and useful to compare the laboratory LC_{50} data with enclosure LC_{50} calculations derived from the 24-h weighted concentrations. With this comparison, the laboratory and enclosure LC_{50} ranges overlap almost completely.

Analysis of the secondary effects studies is not complete and should be considered preliminary. Results of mean weights and lengths from the larval fathead minnow study revealed significant differences related to chlorpyrifos treatment (Table 5). Initially (day −2), mean length and weight were lowest for the control fish, but by day 15 and through day 31 the mean lengths and weights of control fish were significantly greater than fish from the high-treatment enclosures.

Based on the digestive tract content analysis, it appears that a reduction in several chlorpyrifos-sensitive invertebrate forage groups in the treated enclosures contributed to the differences in growth observed between larval fathead minnows from the control and high treatment groups. On day −2 there were no significant differences in the number of prey consumed from any of the eight main forage groups included in this analysis or in the total number of prey consumed between any of four treatment groups (ANOVA, $P > 0.05$). However, by day 7 several significant differences were apparent. More rotifers, chironomids, and hydracarina were consumed in the control enclosures than in some of the treated enclosures, and mean total number of prey con-

Table 4. Juvenile bluegill and fathead minnow percent survival for the first 96 h during the 1986 chlorpyrifos littoral enclosure study

Fish species		Treatment Group			
		Control	Low	Medium	High
Bluegill	mean	100.0a	98.3a	62.2b	0.9c
	SE	0.0	1.7	7.3	0.9
	n*	2	2	4	4
Fathead minnow	mean	100.0	100.0	100.0	99.2
	SE	0.0	0.0	0.0	0.8
	n*	2	2	4	4

Means followed by different letters differ significantly, $P < 0.05$, ANOVA.
*n = number of cages for each treatment group.

Table 5. Mean standard lengths and wet weights of endemic larval fathead minnows sampled on days −2, 7, 15, and 31 relative to chlorpyrifos treatment, 1986 littoral enclosure study

Treatment group	No. measured	Standard length, mm		Wet weight, mg	
		$\bar{x}$	SE	$\bar{x}$	SE
		Day −2			
Control	49	9.46	0.18	4.64	0.31
High	120	9.94	0.09	4.56	0.22
		Day 7			
Control	52	11.57	0.29	9.29	0.80
High	96	10.52	0.17	7.31	0.59
		Day 15			
Control	57	15.59*	0.23	31.54*	1.59
High	84	10.59*	0.22	9.28*	0.80
		Day 31			
Control	38	18.22*	0.45	52.76*	4.47
High	46	13.24*	0.49	22.69*	3.91

*, Denotes where treatment means for a given date and size variable differ significantly, $P < 0.05$, ANOVA.

sumed per fish was also greater in the controls than in the treated enclosures (ANOVA and Tukey's HSD test, $P < 0.05$). By day 15, the number of cladocerans, immature copepods, chironomids, rotifers, hydracarina, and total number of prey consumed per fish were significantly higher in the control enclosures than in the treated enclosures ($P < 0.05$). On day 31, differences between the number of cladocerans and hydracarina consumed per fish in the control enclosures and the treated enclosures were still significant ($P < 0.05$). At no time were any differences found where the number of a given prey type consumed was significantly greater in the treated enclosures than in the control enclosures ($P > 0.05$).

This indicates that foraging opportunities were better in the untreated enclosures and that fish in these enclosures benefited through increased growth. It is important to supplement these digestive tract content data with information on the available forage in the enclosures during the same period. Based on the zooplankton and macroinvertebrate samples that have been processed, it appears that many of the items that were found in reduced numbers after treatment in the digestive tract contents also were reduced in the invertebrate samples.

It is also possible that the growth differences observed between treatment groups were due to chance differences in the densities of larval fish present in the enclosures. Analysis of the larval-density data compiled for the 12 study enclosures indicates that this was probably not the case. There were no significant differences in the mean number of larval fathead minnows or total number of larval fish in any of the 12 enclosures or treatment groups sampled during the study (ANOVA, $P > 0.05$). However, a trend toward lower mean densities in the control enclosures was evident.

The preliminary results of the secondary effects study with introduced green sunfish are less clear than those from the larval fathead minnow study. However, on days 16–17, there was a highly significant trend towards larger mean lengths and weights in the control and low treatment fish as compared to fish from the medium treatment enclosures (ANOVA and Tukey's HSD test, $P < 0.01$). On days 31–32, this trend was still apparent but no longer statistically significant. By days 64–67, fish from the low-treatment enclosures were found to be significantly longer than fish from the medium-treatment enclosures ($P \leq 0.05$) but no other differences between treatment groups were significant. These results indicate the possibility of a secondary effect of the chlorpyrifos treatment on the growth of green sunfish but no conclusions can be made until supplementary data on the diets of these fish following treatment are obtained.

There was a problem associated with the recapture of green sunfish during this study. It was difficult to obtain a uniform and adequate sample size on a precise schedule. This led to unequal sample sizes, increased variance, and difficulties in analyzing and interpreting the data. It seems likely that this would be the case in any free-ranging fish study, especially one involving small fish that are difficult to unobtrusively recapture in littoral enclosures with anything besides a minnow trap. Electro-shocking and hook-and-line recapture are ineffective for fish of the size used in this study, and seining is too destructive to aquatic plants and disruptive to the sediments.

Conclusions

Results from the use of the littoral enclosure protocol to study the environmental chemistry and test the biological effects of the model pesticide chlorpyrifos show the protocol's utility and potential for studying episodic disruptions in natural aquatic systems.

The chlorpyrifos chemistry studies indicated maximum concentrations 1 h after application followed by a rapid decrease in concentration. This profile and the relatively slow vertical mixing rate of the pesticide will allow for more "real-world" laboratory toxicity test designs, as well as a better understanding of the dissipation of a compound in a natural system. Because the chlorpyrifos concentration variability was consistent within treatment levels and the degradation curves were consistent within and between treatments, pesticide concentrations in the littoral enclosures can be accurately replicated for meaningful statistical analyses.

The more significant biological findings include the direct effect of chlorpyrifos on the invertebrate community 4 d after treatments, with the field LOECs apparently more sensitive measures of pesticide toxicity than the laboratory LC_{50} values. Although there were no statistically significant direct effects on the algae community, an increase in algae biomass on day 8 may be attributed to the reduction of herbivorous

invertebrates—an example of an indirect effect of the pesticide application. The relative sensitivities of bluegill sunfish and fathead minnows to chlorpyrifos tested in the laboratory were confirmed with the enclosure tests—an example of field validation capabilities of the protocol. Indirect effects of the pesticide application became apparent with the change in the diets of endemic fathead minnow larvae in the treated enclosures. The reduction of chlorpyrifos-sensitive invertebrates was followed by a significant reduction in growth of the fish.

This protocol will measure the dissipation, mixing, and persistence of a pesticide, provide information on the primary and secondary (ecological) effects on invertebrate and fish populations, and allow a comparison of laboratory toxicity data to actual effects in a natural aquatic system.

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References Cited

Ahmed, W. 1976. The effectiveness of predators of rice field mosquitoes in relation to pesticide use in rice culture. Ph.D. dissertation, University of California, Davis.

Ali, A. & M. S. Mulla. 1978. Declining field efficacy of chlorpyrifos against chironomid midges and laboratory evaluation of substitute larvicides. J. Econ. Entomol. 71: 778–782.

Borthwick, P. W. & G. E. Walsh. 1981. Initial toxicological assessment of Ambush, Bolero, Bux, Dursban, Fentrifanil, Larvin, and Pydrin: static acute toxicity tests with selected estuarine algae, invertebrates and fish. EPA-600/4-81-076. U.S. Environmental Protection Agency, Washington, D.C.

Boyle, T. P., S. E. Finger, F. L. Paulson & D. F. Rabeni. 1985. Comparison of laboratory and field assessment of fluorene-Part II: Effects on the ecological structure and function of experimental pond ecosystems, pp. 134–151. *In* T. P. Boyle [ed.], Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems. ASTM STP 865. American Society for Testing and Materials, Philadelphia.

Brakke, D. F. 1976. Modification of the Whiteside-Williams pattern sampler. J. Fish. Res. Board Can. 33: 2861–2863.

Brazner, J. C., S. J. Lozano, M. L. Knuth, L. J. Heinis, D. A. Jensen, K. W. Sargent, S. L. O'Halloran, S. L. Bertelsen, D. K. Tanner, E. R. Kline & R. E. Siefert. 1988. The effects of chlorpyrifos on a natural aquatic system: a research design for littoral enclosure studies and preliminary data report. Progress report. U.S. Environmental Protecton Agency, Duluth, Minn.

Brazner, J. C., L. J. Heinis & D. A. Jensen. 1989. A littoral enclosure for replicated field experiments. Environ. Toxicol. Chem. 8: 1209–1216.

Brock, D. A. 1977. Comparison of community similarity indices. J. Water Pollut. Control Fed. 49: 2488–2494.

Brooks, J. L. 1959. Cladocera, pp. 587–656. *In* W.T. Edmondson [ed.], Freshwater biology, 3rd ed. Wiley, New York.

Butcher, J. E., M. G. Boyer & C. D. Fowle. 1977. Some changes in pond chemistry and photosynthetic activity following treatment with increasing concentrations of chlorpyrifos. Bull. Environ. Contam. Toxicol. 17: 752–758.

Cooper, W. E. 1965. Dynamics and productivity of a natural population of a freshwater amphipod, *Hyalella azteca*. Ecol. Monogr. 35: 337–394.

Crossland, N. O. & C. J. Wolff. 1985. Fate and biological effects of pentachlorophenol in outdoor ponds. Environ. Toxicol. Chem. 4: 73–86.

deNoyelles, F., Jr., & W. D. Kettle. 1985. Experimental ponds for evaluating bioassay predictions, pp. 91–103. *In* T. P. Boyle [ed.], Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems. ASTM STP 865. American Society for Testing and Materials, Philadelphia.

Dewey, S. L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67: 148–162.

Eaton, J. G., J. Arthur, R. Hermanutz, R. Kiefer, L. Mueller, R. Anderson, R. Erickson, B. Nordling, J. Rogers & H. Pritchard. 1984. Biological effects of Dursban on an outdoor experimental stream ecosystem. Project report. U.S. Environmental Protection Agency, Duluth, Minn.

Edmondson, W. T. 1959. Rotifera, pp. 420–494. *In* W.T. Edmondson [ed.], Freshwater biology, 3rd ed. Wiley, New York.

Federle, P. F. & W. J. Collins. 1976. Insecticide toxicity to three insects from Ohio ponds. Ohio J. Sci. 76: 19–24.

Ferguson, D. E., D. T. Gardner & A. L. Lindley. 1966. Toxicity of Dursban to three species of fish. Mosq. News 26: 80–82.

Giddings, J. M., P. J. Franco, R. M. Cushman, L. A. Hook, G. R. Southworth & A. J. Stewart. 1984. Effects of chronic exposure to coal-derived oil on freshwater ecosystems: II. experimental ponds. Environ. Toxicol. Chem. 3: 465–489.

Hamilton, M. A., R. C. Russo & R. V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Tech. 11: 714. Correction, 1978, 12: 417.

Helson, B. V., G. A. Surgeoner & W. E. Ralley. 1979. Susceptibility of *Culex* spp. and *Aedes* spp. larvae (Diptera: Culicidae) to temephos and chlorpyrifos in southern Ontario. Proc. Entomol. Soc. Ont. 110: 79–83.

Hester, F. E. & J. A. Dendy. 1962. A multi-plate sampler for aquatic macroinvertebrates. Trans. Am. Fish. Soc. 91: 420–421.

Holcombe, G. W., G. L. Phipps & D. K. Tanner. 1982. The acute toxicity of Kelthane, Dursban, Disulfoton, Pydrin, and Permethrin to fathead minnows *Pimephales promelas* and rainbow trout *Salmo gairdneri*. Environ. Pollut. Ser. A Ecol. Biol. 29: 167–178.

Hughes, D. N., M. G. Boyer, M. H. Papst, C. D. Fowle, G. A. Reese & P. Baulu. 1980. Persistence of three organophosphorus insecticides in artificial ponds and some biological implications. Arch. Environ. Contam. Toxicol. 9: 269–279.

Hurlbert, S. H., M. S. Mulla, J. O. Keith, W. E. Westlake & M. E. Dusch. 1970. Biological effects and persistence of Dursban in freshwater ponds. J. Econ. Entomol. 63: 43–52.

Hurlbert, S. H., M. S. Mulla & H. R. Wilson. 1972. Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations on freshwater ponds. Ecol. Monogr. 42: 269–299.

Jarvinen, A. W. & D. K. Tanner. 1982. Toxicity of selected controlled release and corresponding unformulated technical grade pesticides to the fathead minnow *Pimephales promelas*. Environ. Pollut. Ser. A. Ecol. Biol. 27: 179–195.

Jarvinen, A. W., B. R. Nordling & M. E. Henry. 1983. Chronic toxicity of Dursban (chlorpyrifos) to the fathead minnow (*Pimephales promelas*) and the resultant acetylcholinesterase inhibition. Ecotoxicol. Environ. Saf. 7: 423–434.

Jarvinen, A. W., D. K. Tanner & E. R. Kline. 1988. Toxicity of chlorpyrifos, endrin or fenvalerate to fathead minnows following episodic or continuous exposure. Ecotoxicol. Environ. Saf. 15: 78–95.

Johnson, W. W. & M. T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Research Publication 137. U.S. Fish and Wildlife Service, Columbia, Mo.

Kaushik, N. K., G. L. Stephenson, K. R. Solomon & K. E. Day. 1985. Impact of permethrin on zooplankton communities in limnocorrals. Can. J. Fish. Aquat. Sci. 42: 77–85.

Larsen, D. P., F. deNoyelles, Jr., F. Stay & T. Shiroyama. 1986. Comparisons of single-species, microcosm and experimental pond responses to atrazine exposure. Environ. Toxicol. Chem. 5: 179–190.

Levy, R. & T. W. Miller, Jr. 1978. Tolerance of the planarian *Dugesia dorotocephala* to high concentrations of pesticides and growth hormones. Entomophaga 23: 31–34.

Macek, K. J., D. F. Walsh, J. W. Hogan & D. D. Holz. 1972. Toxicity of the insecticide Dursban to fish and aquatic invertebrates in ponds. Trans. Am. Fish. Soc. 101: 420–427.

Maly, M. & E. Ruber. 1983. Effects of pesticides on pure and mixed species cultures of salt marsh pool algae. Bull. Environ. Contam. Toxicol. 30: 464–472.

Marshall, W. K. & J. R. Roberts. 1978. Ecotoxicology of chlorpyrifos. National Research Council of Canada, Associate Committee on Scientific Criteria, NRCC 16079. 314 pp.

Papst, M. H. & M. G. Boyer. 1980. Effects of two organophosphorus insecticides on the chlorophyll a and pheopigment concentrations of standing ponds. Hydrobiologia 69: 245–250.

Phipps, G. L. & G. W. Holcombe. 1985. A method for aquatic multiple species toxicant testing: acute toxicity of 10 chemicals to 5 vertebrates and 2 invertebrates. Environ. Pollut. Ser. A Ecol. Biol. 38: 141–157.

Renkonen, O. 1938. Statisch-kologiische untersuchungen uber die terrestrische Kaferwelt der Finnischen Bruchmoore. Ann. Zool. Soc. Zool. Bot. Fenn. 'Vanamo' 6: 1–231.

Rettich, F. 1977. The susceptibility of mosquito larvae to eighteen insecticides in Czechoslovakia. Mosq. News 37: 252–257.

Roberts, D. & T. A. Miller. 1970. The effects of diatoms on the larvicidal activity of Dursban. November 1969–March 1970. National Technical Information Service, AD-724, Entomological Special Study 31-002-71, Springfield, Va.

Ruber, E. & J. Baskar. 1969. Sensitivities of selected microcrustacea to eight mosquito toxicants, pp. 99–103. *In* Proceedings, 55th Annual Meeting New Jersey Mosquito Exterminators Association, Trenton, N. J.

Saleh, M. S., I. A. Gaaboub & M. I. Kassem. 1981. Larvicidal effectiveness of three controlled release formulations of Dursban and dimilin on *Culex pipiens* and *Aedes aegypti*. J. Agric. Sci. 97: 87–96.

Sanders, H. O. 1969. Toxicity of pesticides to the crustacean *Gammarus lacustris*. Technical Paper 25. U.S. Fish and Wildlife Service, Columbia, Mo.

1972. Toxicity of some insecticides to four species of malacostracan crustaceans. Technical Paper 66. U.S. Fish and Wildlife Service, Washington, D.C.

Sanders, H. O. & O. B. Cope. 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13: 112–117.

SAS Institute. 1982. SAS user's guide: statistics. SAS Institute, Cary, N.C.

Sharom, M. S. & K. R. Solomon. 1981. Adsorption and desorption of permethrin and other pesticides on glass and plastic materials used in bioassay procedures. Can. J. Fish. Aquat. Sci. 38: 199–204.

Siefert, R. E., R. L. Anderson, A. W. Jarvinen, C. F. Kleiner, N. Larson, L. H. Mueller, B. R. Nordling, D. K. Tanner & J. A. Zischke. 1984. Effects of Dursban (chlorpyrifos) on non-target aquatic organisms in a natural pond undergoing mosquito control treatment. Progress report. U.S. Environmental Protection Agency, Duluth, Minn.

Siefert, R. E., R. L. Anderson, J. Helgen, C. F. Kleiner, N. Larson, B. Nordling, D. K. Tanner & J. A. Zischke. 1986. Effects of Abate (temephos) on non-target aquatic organisms in a natural pond undergoing mos-

quito control treatment. Progress report. U.S. Environmental Protection Agency, Duluth, Minn.

Siefert, R. E., R. L. Anderson, J. C. Brazner, L. J. Heinis, D. A. Jensen & M. L. Knuth. 1987. Effects of Dursban (chlorpyrifos) on aquatic organisms in enclosures in a natural pond. Final report. U.S. Environmental Protection Agency, Duluth, Minn.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry, 2nd ed. Freeman, New York.

Solomon, K. R., J. Y. Yoo, D. Lean, N. K. Kaushik, K. E. Day & G. L. Stephenson. 1985. Dissipation of permethrin in limnocorrals. Can. J. Fish. Aquat. Sci. 42: 70–76.

1986. Methoxychlor distribution, dissipation, and effects in freshwater limnocorrals. Environ. Toxicol. Chem. 5: 577–586.

Stephenson, R. R. & D. F. Kane. 1984. Persistence and effects of chemicals in small enclosures in ponds. Arch. Environ. Contam. Toxicol. 13: 313–326.

Stephenson, G. L., N. K. Kaushik, K. R. Solomon & K. Day. 1986. Impact of methoxychlor on freshwater communities of plankton in limnocorrals. Environ. Toxicol. Chem.: 587–603.

Stokes, P. M. 1984. pH-related changes in attached algal communities of softwater lakes, pp. 43–61. *In* G. E. Hendrey [ed.], Early biotic responses to advancing lake acidification. Ann Arbor Science, Boston.

Strong, D. R., Jr. 1973. Amphipod amplexus, the significance of ecotypic variation. Ecology 54: 1383–1388.

U.S. Environmental Protection Agency. 1983a. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Method 120.1. Cincinnati, Ohio.

1983b. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Method 150.1. Cincinnati, Ohio.

1983c. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Method 310.1.

1983d. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Method 110.2. Cincinnati, Ohio.

1983e. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. Method 180.1. Cincinnati, Ohio.

1983f. Methods for chemical analysis of water and wastes. EPA-600/4-79-020, Method 415.1. Cincinnati, Ohio.

1984. Methods for chemical analysis of water and wastes. EPA-600/4-84-017, Method 300.0. Cincinnati, Ohio.

Wauchope, R. D. 1978. The pesticide content of surface water draining from agricultural fields—a review. J. Environ. Qual. 7: 459–471.

Wetzel, R. G. & G. E. Likens. 1979. Limnological analysis. Saunders, Philadelphia.

Whiteside, M. C. 1974. Chydorid (Cladoceran) ecology: seasonal patterns and abundance of populations in Elk Lake, Minnesota. Ecology 55: 538–550.

Whiteside, M. C. & J. B. Williams. 1975. A new sampling technique for aquatic ecologists. Verh. Int. Ver. Limnol. 19: 1534–1539.

Wilson, M. S. 1959. Calanoida, pp. 795–815. *In* W. T. Edmondson [ed.], Freshwater biology, 3rd ed. Wiley, New York.

Yeatman, H. C. 1959. Cyclopoids, pp. 795–815. *In* W. T. Edmondson [ed.], Freshwater biology, 3rd ed. Wiley, New York.

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